REMARKS

In the Office Action dated February 23, 2004, claims 1-4, 6-9 and 11-32 are pending and under consideration. Claims 21 and 28 are objected to as dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claim 24 is objected to for certain alleged informalities. Claims 2, 7, 18 and 25 are rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Claim 32 is rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Claims 1-4, 6-9, 11-12, 13-20, 22-27 and 29-32 are rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enabling support. Claims 1-2, 6-7, 11-12, 24-25, 30-31, and 32 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Conrad et al. (U.S. Patent 5,707,617). Claims 1-4, 6-9, 13-16, 17-20 and 32 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Lindsay et al. (*American J. Vet. Res.* 56: 1176-1180, Sept. 1995). Claims 1-3, 13-15 and 17-19 are rejected under 35 U.S.C. §102(a) as allegedly anticipated by Lindsay et al (*American J. Vet. Res.* 57: 68-72, January 1996). Claims 1-4 and 6-9 are rejected under 35 U.S.C. $102(a) as allegedly anticipated by Hemphill (*Infection and Immunity* 64: 4279-4287, October 1996). Claims 1-2, 6-7, 13-14 and 17-18 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Kim et al. (U.S. Patent 5,976,553).

This Response addresses each of the Examiner's rejections. Applicants therefore respectfully submit that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim 24 is objected to for employing both active and passive tense. The Examiner indicates that amending the claim to recite positive characteristics and the combination of components could obviate this objection.

Claim 24 has been amended in accordance with the Examiner's suggestion. Withdrawal of the objection is therefore respectfully requested.

Claims 2, 7, 18 and 25 are rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. The Examiner objects to the recitation "temperature-sensitive". The Examiner contends that this phrase does not define how the temperature-sensitive strains differ structurally from the strains of the independent claims. The Examiner is of the opinion that all strains of *Neospora* are considered to be temperature sensitive to some extent.

In response to the Examiner's objection, Applicants have amended claims 2, 7, 18 and 25 to further define the cells as having "a reduced growth rate at the body temperature of said mammal compared to a temperature lower than the body temperature of said mammal." Support for such amendment is found in the specification, e.g., at page 5, lines 24-27. Applicants further submit that the cells recited in the independent claims are not necessarily temperature-sensitive. It is clear to those skilled in the art that temperature-sensitive cells are presumably different in structure (i.e., certain gene sequence(s)) from cells that are not temperature-sensitive. It is respectfully submitted that the claims, as presently amended, are not indefinite. Withdrawal of the rejection of claims 2, 7, 18 and 25 is therefore respectfully requested.

Claim 32 is rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. The Examiner contends that it is not clear whether the phrase "one or more other antigens" is referring to a non-specific antigenic adjuvant, an additional strain of cells, or additional vaccine antigens for a different pathogen.

Applicants have amended claim 32 to recite "one or more antigens other than a *Neospora* antigen". It is respectfully submitted that claim 32, as amended, is not indefinite. Withdrawal of the rejection of claim 32 is therefore respectfully requested.

Claims 1-4, 6-9, 11-12, 13-20, 22-27 and 29-32 are rejected under 35 U.S.C. §112, first paragraph.

The Examiner acknowledges that the specification is enabling for compositions that induce an immune response against *Neospora* and other additional immunogens. The Examiner contends that the specification does not provide enablement for the use of compositions that induce a protective immune response based upon any type of attenuation, nor enabling for the treatment of pre-existing infection by *Neospora* or for prevention of *Neospora* infection.

The Examiner contends that one skilled in the art would not reasonably predict whether the utilization of any attenuated strain would induce protective immunity in a mammal. In support of her position, the Examiner has also cited several references, including Andrianarivo et al. (*International Journal of Parasitology* 30: 985-990, 2000), Anderson et al. (*Animal Reproduction Science* 60-61: 417-431, 2000, abstract) and Nishikawa et al. (*Clinical Diag. Lab. Immunity* 8: 811-817, 2001). The Examiner further alleges that the specification does not provide substantive evidence in support of the claimed vaccines. Moreover, the Examiner alleges that the specification fails to provide an adequate written description of what mutations and attenuations would result in a vaccine strain of *Neospora*. Thus, the Examiner concludes that it would take undue experimentation for one skilled in the art to formulate and use a successful vaccine as presently claimed.

With respect to the references cited by the Examiner, Applicants respectfully submit that the disclosures of the references are not relevant to assessing the enablement of the claimed invention. Specifically, Applicants observe that Andrianarivo et al. merely disclose a killed *Neospora* vaccine, which failed to induce protective immunity. The culture-derived *Neospora* tachyzoites administered to heifers in Andrianarivo et al., which the Examiner has referenced,

are merely the infectious challenge materials which Andrianarivo et al. employed to test the efficacy of the killed vaccine. Therefore, the failure of the killed *Neospora* vaccine disclosed by Andrianarivo et al. is not relevant to the attenuated *Neospora* vaccine, as presently claimed.

Furthermore, Applicants observe that the abstract of Anderson et al. alleges a lack of proven protocol for the prevention or treatment of neosporosis. However, Applicants respectfully submit that the disclosure of Anderson et al. focused on the pathology of *Neospora* infection, not the vaccines against neosporosis. It is not clear whether Anderson et al. were even aware of attenuated *Neospora* vaccines.

Applicants further observe that Nishikawa et al. simply disclose that a mammal that had previously been infected with *Neopsora caninum* became reinfected with chronic infection. Applicants respectfully submit that the failure of a prior infection in inducing protective immunity is by no means an indication of the efficacy of a vaccine composition made of an effective amount of attenuated *Neopsora* cells, as presently claimed.

Applicants respectfully submit that the present specification provides adequate teaching for those skilled in the art to prepare attenuated, protective strains of *Neospora* for use as vaccines. The Examiner's attention is respectfully directed to the specification, particularly in the section entitled "Preparation of Attenuated Strains of Neospora" on pages 5-11. This portion of the specification adequately describes a variety of methods by which attenuated strains of *Neospora* can be prepared, including by exposing *Neospora* cells to a mutagen (chemical or radiation), or by known recombinant techniques. The specification (page 9, line 26 to page 10, line 5) describes how an attenuated strain may be selected and characterized. The specification also provides examples of attenuated, temperature-sensitive strains of *Neospora*, including NCTS-4, NCTS-8 and NCTS-12. See pages 15-27 of the specification. The specification further

describes, particularly in the section entitled "Preparation and Use of Vaccines" on page 11-14, a variety of methods by which vaccine formulations can be prepared and administered using an attenuated strain of *Neospora* prepared according to the invention. In addition, the specification illustrates how vaccination with live cells of NCTS-8 protected mice (pages 27-33) and goats (pages 35-38) against disease caused by infection with pathogenic *Neospora*.

As to the Examiner's contention that the specification fails to provide an adequate written description of what mutations and attenuations would result in a vaccine strain of *Neospora*, Applicants respectfully submit that the skilled artisan need not always identify the specific mutation in an attenuated strain of *Neospora* to use the strain in a vaccine composition. As submitted above, the present specification provides adequate description for the preparation of an attenuated strain of *Neospora*, as well as methods of determining the attenuated pathogenicity of a strain of *Neospora*, and methods of determining the capacity of an attenuated strain to protect an animal against infection with pathogenic *Neospora*. In addition to these teachings, the specification provides specific exemplification of attenuated strains of *Neospora* (such as NCTS-4, NCTS-8 and NCTS-12) and a vaccine composition containing such an attenuated strain (NCTS-8). Therefore, it is respectfully submitted that the vaccine compositions as presently claimed are adequately described in the specification.

In further support of Applicants' position that the claimed subject matter is fully enabled and adequately described in the specification, Applicants respectfully direct the Examiner's attention to the Declaration of David A. Brake. Ph.D. (the "Brake Declaration"), submitted in the grand-parent application, Serial No. 09/260,414, which describes, *inter alia*, two new attenuated, protective strains (*i.e.*, strains 4D8 and 6H11), which were readily prepared

using the guidance provided in Applicants' disclosure. A copy of the Brake Declaration is enclosed herewith as **Exhibit 1**.

As described in Paragraphs 6-9 of the Brake Declaration, novel, mutagenized strains of *Neospora* were prepared by the same chemical mutagenesis procedure used to prepare strain NCTS-8 exemplified in the present specification. However, instead of using *Neospora caninum* strain NC-1 as the source material, a distinctly different strain, NC-2, was used. From three rounds of the mutagenesis procedure using NC-2 as a source strain, a total of 19 positive clones were identified as potentially efficacious based on their ability to grow at 32°C. As explained in the Brake Declaration (¶ 10), these novel clones were tested for protective ability in an "adoptive transfer" experiment, where survival curve data (Graphs 1 and 2, Exhibits C and D, attached to the Brake Declaration) demonstrate that several of these novel strains, including strains 4D8 and 6H11, were capable of increasing the survival of the recipient SCID/bg mouse after NC-1 challenge, *i.e.*, these strains demonstrate an ability to protect.

As further explained in the Brake Declaration (¶ 11), at least 6 novel strains prepared from parental strain NC-2 were able to trigger protective immunity in mammals against neosporosis, similar to NCTS-8 prepared from NC-1. Those 6 novel strains, designated as strains "2E4", "2F11", "3G4", "4D8", "6G7", and "6H11", were selected for further analysis in an art-accepted mouse neosporosis model system to determine whether they also exhibited reduced pathogenicity (*i.e.*, attenuation).

Paragraphs 12, 13 and 14 of the Brake Declaration describe experiments demonstrating how strains 4D8 and 6H11 were shown to be attenuated. Strain 4D8 was shown to be attenuated by: (i) a significantly increased survival rate (¶ 12); (ii) a lack of histochemical lesions after infection (¶ 13); and (iii) a reduced growth rate at both 32°C and the target body

temperature of 40°C (¶ 14) (see Brake Declaration, Tables 1 and 2, and Exhibits E-G attached thereto). Strain 6H11 was shown to be attenuated by a significantly increased survival rate (¶ 12).

Therefore, Applicants successfully employed the teaching of their own disclosure to readily prepare two additional strains of *Neospora*, *i.e.*, strains 4D8 and 6H11; 4D8 and 6H11 exhibit attenuated pathogenicity compared to their parent strain (NC-2), and can trigger an immune response that protects a mammal against neosporosis when administered as a live vaccine. Accordingly, Applicants respectfully submit that the instant disclosure provides one skilled in the art with adequate guidance with which to prepare additional attenuated, protective strains of *Neospora* without undue experimentation. Applicants should not be required to limit the claims only to the attenuated strains exemplified in the application as filed, since those strains are merely examples of what can be accomplished using Applicants' invention.

In view of the ample guidance provided by the specification, Applicants respectfully submit that the application as filed clearly teaches one skilled in the art how to obtain the claimed vaccine compositions without undue experimentation. Applicants submit that the rejection of claims 6-12 under 35 U.S.C. §112, first paragraph, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-2, 6-7, 11-12, 24-25 and 30-32 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Conrad et al. (U.S. Patent No. 5,707,617).

Applicants observe that the Conrad patent is primarily directed to two pathogenic bovine isolates of *Neospora* (BPA1 and BPA2), characterized by their isolation, pathology, immunology in host animals and selected nucleotide sequences. The Conrad patent does not

teach or suggest attenuated strains derived from these pathogenic isolates, let alone attenuated strains that are capable of inducing protective immunity in a mammal, as presently claimed.

In this regard, Applicants respectfully submit that the instant claims recite "cells of an isolated strain derived from a pathogenic parent strain of a species of *Neospora*", which cells exhibit attenuated pathogenicity and are capable of triggering an immune response that protects a mammal against neosporosis when administered as a live vaccine. The present specification therefore teaches how to isolate a strain having attenuated pathogenicity relative to the parent strain. For example, at page 9, lines 26-32, the specification provides that, after the attenuation step, cells that exhibit one or more indicators of attenuated pathogenicity are selected from the culture and clonally propagated after limiting dilution.

In contrast, the Conrad patent does not teach or suggest isolated attenuated strains derived from pathogenic *Neospora* isolates, or how to obtain such strains. The only mention in the Conrad patent relating to attenuated *Neospora* appears at column 11, lines 34-47, where Conrad states that "[an] attenuated *Neospora* vaccine can only be used in the absence of a risk of human infection should the milk or tissues of immunized animals be consumed." It is respectfully submitted that such disclosure by Conrad et al. by no means constitutes teaching of isolated attenuated strains of *Neospora* or how to obtain such strains.

Applicants respectfully submit that, since the Conrad patent does not teach any isolated attenuated strain, the Conrad patent could not have possibly taught an attenuated strain that is temperature-sensitive, much less a strain that is capable of triggering a protective immune response in a mammal when administered as a live vaccine. To Applicants' knowledge, Applicants are the first to provide successful live cultures of cells of an isolated strain derived from a pathogenic *Neospora* parent strain, which are attenuated and protective as a live vaccine,

despite the fact that neosporosis has been recognized as a serious disease in animals, particularly in cattle and dogs, since the mid-1980's.

In view of the foregoing, Applicants respectfully submit that the Conrad patent does not teach the claimed invention. The rejection under 35 U.S.C. §102(e) based on Conrad et al., is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-4, 6-9, 13-16, 17-20 and 32 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Lindsay et al. (*American J. Vet. Res.* 56: 1176-1180, Sept. 1995).

Lindsay et al. disclose a composition of cultured *N. caninum* cells that were maintained by subinoculation into noninfected monolayers every 3 to 4 days. The cultures were used to formulate a sterile composition of cells adjusted to 10^7 in 3 ml of pharmaceutical carrier. The Examiner contends that the subcultured cells disclosed by Lindsay (1995) would be sensitive to freezing temperatures and would also be attenuated through one or more passages in *in vitro* culture. Thus, the Examiner contends that Lindsay et al. inherently anticipate the claimed compositions and methods.

The Examiner also contends that Lindsay et al. disclose a combination composition that comprises several different strains of *N. caninum* cells, specifically NC-1, NC-2 and NC-3. The combination composition was formulated as an inoculum and was administered intramuscularly or intravenously.

Applicants respectfully submit that, although Lindsay et al. (1995) disclose *in vitro* cultures of *N. caninum* cells, there is no teaching in Lindsay et al. that the cultures exhibit attenuated pathogenicity as compared to the parent pathogenic *N. caninum* strain. Further, Lindsay et al. (1995) does not teach or suggest an isolated strain that exhibits attenuated pathogenicity or how to obtain such a strain, as presently claimed. It is true that attenuation of a

pathogenic *Neospora* parent strain can be achieved by *in vitro* passaging. However, this does not mean that culturing a pathogenic *Neospora* strain *in vitro* alone, i.e., absent selection and cloning, results in an isolated attenuated strain. An *in vitro* culture initiated with a pathogenic parent strain can contain a mixture of cells, heterogeneous in their pathogenic characteristics.

In contrast, the present claims are directed to live cells of an isolated strain derived from a pathogenic parent strain of a species of *Neospora*, and to methods of preparing such strain. The present specification teaches how to attenuate a pathogenic parent strain of *Neospora* and subsequently isolate a strain having attenuated pathogenicity. For example, at page 9, lines 26-32, the specification provides that, after the attenuation step, cells that exhibit one or more indicators of attenuated pathogenicity are selected from the culture and clonally propagated after limiting dilution. See also page 15, lines 23-29, Example 1 of the specification, where three temperature-sensitive *Neospora* clones were isolated; and page 15, line 30 through page 21, line 11, where the attenuated pathogenicity of these clones were determined.

Applicants further submit that the Lindsay et al. (1995) reference does not teach any isolated attenuated strains that are temperature-sensitive. More specifically, the Lindsay et al. (1995) reference does not teach attenuated strains that exhibit reduced growth at the body temperature of a mammal, e.g., 37°C, as compared to a temperature lower than the body temperature.

In view of the foregoing, Applicants respectfully submit that the Lindsay et al. (1995) reference does not teach the claimed invention. Withdrawal of the rejection based on Lindsay et al. (1995) is therefore respectfully requested.

Claims 1-3, 13-15 and 17-19 are rejected under 35 U.S.C. §102(a) as allegedly anticipated by Lindsay et al (*American J. Vet. Res.* 57: 68-72, January 1996).

Lindsay et al. (1996) disclose the production of mutated strains of *Neospora caninum* through treatment with N-methyl-N-nitrosoguanidine. The Examiner contends that the mutated strains would be attenuated and temperature sensitive at least to freezing temperatures, and therefore, the cultured *N. caninum* cells of Lindsay et al. anticipate the now claimed invention.

Applicants respectfully submit that there is no teaching in Lindsay (1996) that the mutated *Neospora* cells exhibit attenuated pathogenicity as compared to the parent pathogenic strain. The mutants could have the same or stronger pathogenicity as compared to the parent pathogenic strain. Furthermore, there is no teaching in Lindsay (1996) that the two *Neospora* mutants are temperature-sensitive, i.e., characterized by a reduced growth at the body temperature of a mammal as compared to a lower temperature. Furthermore, there is no teaching in Lindsay (1996) that the *Neospora* mutants are capable of triggering an immune response in a mammal against neosporosis. The fact that a certain characteristic may be present in the prior art is not sufficient to establish the inherency of that result of characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (emphasis added).

Accordingly, it is respectfully submitted that Lindsay et al. (1996) do not teach an isolated, attenuated strain derived from a pathogenic parent strain of a species of *Neospora*, that is capable of triggering a protective immune response in a mammal, as presently claimed. Nor do Lindsay et al. teach a method of preparing such a strain. Thus, the rejection under 35 U.S.C. §102(a) based on Lindsay et al. (January 1996) is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-4 and 6-9 are rejected under 35 U.S.C. $102(a) as allegedly anticipated by Hemphill (*Infection and Immunity* 64: 4279-4287, October 1996).

Hemphill discloses a culture of Vero cells (monkey kidney cells) that comprises NC-1 *Neospora*. The cells were maintained in culture and passaged at least once a week. The cells, together with a pharmaceutically acceptable carrier (phosphate buffered saline), were used to produce a composition. The Examiner contends that the cultured NC-1 *Neospora* in Monkey kidney cells would be attenuated through the one or more *in vitro* passages, and would also be sensitive to freezing temperature. Therefore, the Examiner concludes that Hemphill anticipates the claimed invention.

Applicants respectfully submit that there is no teaching in Hemphill that the cultured *Neospora* cells exhibit attenuated pathogenicity as compared to the parent pathogenic strain. Nor is there any teaching in Hemphill of an isolated attenuated *Neospora* strain, much less an isolated attenuated *Neospora* strain that is temperature-sensitive as presently defined. The *in vitro* culture of cells disclosed by Hemphill is a merely mixture of cells which can be hetergeneous in their pathogenic characteristics. Moreover, there is no teaching in Hemphill of an isolated attenuated Neospora strain capable of triggering a protective immune response in a mammal. In addition, Hemphill does not teach how to obtain the strains, as presently claimed.

Accordingly, it is respectfully submitted that Hemphill does not teach the claimed compositions or methods. Thus, the rejection under 35 U.S.C. §102(a) based on Hemphill is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-2, 6-7, 13-14 and 17-18 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Kim et al. (U.S. Patent No. 5,976,553).

The Examiner alleges that Kim et al. disclose and claim an attenuated strain of *Neospora* (see claim 4) that has been attenuated through transformation with a specific DNA sequence. The Examiner contends that the strains would be sensitive to freezing temperatures.

Thus, the Examiner concludes that the attenuated strain of *Neospora* disclosed by Kim et al. anticipates the claimed invention. The Examiner further alleges that Kim et al. also disclose the instant methods of preparing an attenuated strain.

Applicants respectfully submit that Kim et al. teach and claim a parasite strain transformed with an exogenous DNA (see claim 4). However, a transformed parasite strain is not necessarily attenuated in its pathogenicity as compared to the parent strain.

Kim et al. also disclose that the transformation procedure described therein provides means to prepare an attenuated parasite strain in general, and that disruption of one or more genes in *Toxoplasma* may allow the generation of a strain which could not give rise to chronic infections. However, there is no teaching in Kim et al. of an attenuated *Neospora* strain or how a *Neospora* strain can be attenuated. One skilled in the art would not reasonably expect that the teachings provided by Kim et al. with respect to attenuating *Toxoplasma* would be successfully applied to *Neospora*. In addition, there is no teaching in Kim et al. of an attenuated strain, temperature-sensitive or not, that is capable of triggering an immune response in a mammal.

Accordingly, it is respectfully submitted that Kim et al. do not teach the claimed invention. The rejection under 35 U.S.C. §102(e) based on Kim et al. is therefore overcome. Withdrawal of the rejection is respectfully requested.

Claim 1-4 and 6-9 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 of U.S. Patent No. 6,656,479. The Examiner states that, although the conflicting claims are not identical, they are not patentably distinct from each other.

Applicants respectfully submit that a terminal disclaimer will be filed in due course to overcome the obviousness-type double patenting rejection.

In view of the foregoing, it is respectfully submitted that the present case is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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Enc.: Exhibit 1: The Brake Declaration (with Exhibits A-G attached thereto)